STUDIES IN SKIN METABOLISM: UTILIZATION OF KETO-ACIDS BY RAT SKIN HOMOGENATES*

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Metabolic studies of the skin have, for the most part, been confined to respiration studies using various substrates. The possibility that the tricarboxylic acid cycle is operative in the skin has been postulated in spite of the fact that the respiration studies showed that certain of the acids of the cycle did not stimulate respiration, or did so only slightly, and only at high concentrations (1–3).

Recently, Pomerantz *et al.* (4), using C^{14} labeled glucose in incubation with whole skin from young rats, has obtained further evidence for the existence of the tricarboxylic acid cycle in skin. In addition, Halprin and Chow (5), using C^{14} labeled acetate in perfused dog skin, have presented additional evidence for the existence of this cycle in skin. The relatively high concentrations of labeled succinate isolated by these latter authors, combined with the previous reported observations concerning lack of stimulation by certain acids of the citric acid cycle, have led to the postulation that, in addition to the citric acid cycle, an alternative metabolic pathway (glyoxylate shunt) may also exist. It is the purpose of this communication to present evidence showing the utilization of glyoxylate by epidermis.

EXPERIMENTAL

Homogenates of rat epidermis was prepared in the cold using Tris buffer (pH 7.35). Cellular debris was spun out, and the supernatant was used in incubation mixtures.

Incubations were carried on in the Dubnoff metabolic shaker‡ at 30° C. Each incubation mixture was of sufficient quantity so that 1 ml. aliquots could be removed at various time intervals. The aliquot of the incubation mixture removed immediately after the addition of the homogenate, or in experiments where ATP was necessary, the addition of ATP to the incubation mixture, was designated as zero time. At the various time intervals, 1 ml. aliquots of the incubation mixture were added to 0.2 ml. of 30% trichloracetic acid, and 0.4 ml. of water added. The precipitated protein was eliminated by centrifuging and decanting the supernatant. Determinations were carried out on the supernatant.

Keto acid determinations were carried out by a modification of the Friedemann and Haugen method (6) for determination of keto acids in blood and urine.

RESULTS

Keto Acid Utilization in Rat Skin Homogenates

The complete system contained:
600 μ moles of Tris buffer (pH 7.35)
6 08 μ moles of glyoxylate
0.49 μ moles of CoA
2.36 μ moles DPN
2.40 μ moles TPN
13 μ moles glutathione
250 μ moles KCl
25 μ moles $MgCl_2$
2.73 μ moles ATP
1 ml. of skin homogenate in a total volume of 6 ml.

The results are presented in Table I.

In similar experiments using glyoxylate as substrate with added acetate, the disappearance of keto acid was identical with and without acetate.

TABLE I

Time Minutes	Keto Acid Disappearance Expressed as Optical Density at 520 mμ			
	Glyoxylate	Pyruvate	Oxalo-acetate	α-keto-glutarate
0	0.440	0.90	0.74	0.78
15	0.430	0.95	0.62	0.74
30	0.395	0.77	0.50	0.65
45	0.390	0.66	0.43	0.58
60	0.345	0.66	0.40	0.54

DISCUSSION

The utilization of both glyoxylate and α-ketoglutarate by the rat skin homogenates gives further evidence that the tricarboxylic acid cycle and the glyoxylate shunt may both be operative in skin. However, additional experiments will be needed to establish whether the glyoxylate which is disappearing is being metabolized via the glyoxylate shunt mechanism.

The classical tricarboxylic acid cycle is a catabolic one in that for each 2 carbon atoms entering the cycle as acetate, 2 are released as carbon dioxide. On the other hand, the glyoxylate shunt is an anabolic cycle in which carbon atoms are not lost as carbon dioxide. This cycle may thus be serving in the skin as a means of interconversion of fats, carbohydrates, and proteins without loss of carbon atoms from the cycle.

SUMMARY

Evidence has been presented showing that glyoxylate, pyruvate, oxaloacetate, and α-keto-

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glutarate are utilized by rat skin homogenates. It is possible that both the tricarboxylic acid cycle and the glyoxylate shunt may be operative in skin.

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